ANBCA2

ANALYTICAL BIOCHEMISTRY

An International Journal of
Methods in the Biological Sciences

EDITOR-IN-CHIEF

William B. Jakoby

EXECUTIVE EDITORS

Assistant to the Editor-in-Chief, William S. Allison

Shelby L. Berger
Ludwig Brand
Peter Brodelius
Wybren de Jong
Jack E. Dixon
Theodore Friedmann
Othmar Gabriel
William F. Harrington
Tony E. Hugli
Richard D. Klausner
John H. Law
Y. C. Lee
Walter M. Lovenberg
William D. McElroy
Sherie L. Morrison
Richard N. Perham
James F. Riordan
Enrique Rozengurt
J. Edwin Seegmiller
Clarence H. Suelter
Bert L. Vallee
Helen Van Vunakis
Arthur Weissbach

Alvin Nason, Founding Editor, 1960–1977
Nathan O. Kaplan and William D. McElroy,
Editorial Committee Co-Chairmen, 1978–1986

Volume 170, 1988

ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
San Diego New York Boston
London Sydney Tokyo Toronto

Copyright © 1988 by Academic Press, Inc.

All Rights Reserved

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the copyright owner.

The appearance of the code at the bottom of the first page of an article in this journal indicates the copyright owner's consent that copies of the article may be made for personal or internal use, or for the personal or internal use of specific clients. This consent is given on the condition, however, that the copier pay the stated per copy fee through the Copyright Clearance Center, Inc. (27 Congress Street, Salem, Massachusetts 01970), for copying beyond that permitted by Sections 107 or 108 of the U. S. Copyright Law. This consent does not extend to other kinds of copying, such as copying for general distribution, for advertising or promotional purposes, for creating new collective works, or for resale. Copy fees for pre-1988 articles are as shown on the article title pages; if no fee code appears on the title page, the copy fee is the same as for current articles.

0003-2697/88 $3.00

MADE IN THE UNITED STATES OF AMERICA

CONTENTS OF VOLUME 170

NUMBER 1, APRIL 1988

Electroblotting onto Glass-Fiber Filter from an Analytical Isoelectrofocusing Gel: A Preparative Method for Isolating Proteins for N-Terminal Microsequencing
Jyh-Cheng Hsieh, Fu-Pang Lin, and Ming F. Tam 1

A One-Step Preparative Method for Separating SER 6-Phosphorylated HMG 14 from Unphosphorylated HMG 14 and *in Vitro* Phosphorylation Reaction Components
Diane P. Bofinger, Nancy W. Fucile, and Stephen W. Spaulding 9

Microsequence Analysis of Peptides and Proteins. VIII. Improved Electroblotting of Proteins onto Membranes and Derivatized Glass-Fiber Sheets
Qin-Yu Xu and John E. Shively 19

A General Procedure for Screening Inhibitory Antibodies: Application for Identifying Anti-Protein Kinase C Antibodies
Daria Mochly-Rosen and Daniel E. Koshland, Jr. 31

Transformation of Bacteria with Plasmid DNA by Electroporation
Stefan Fiedler and Reinhard Wirth 38

Assays for Malonyl-Coenzyme A Synthase *Yu Sam Kim and Son Kwon Bang* 45

Protein-Binding Capacity of Microquantities of Tannins
Rajinder K. Dawra, Harinder P. S. Makkar, and Bhupinder Singh 50

Monosaccharide Analysis of Glycoconjugates by Anion Exchange Chromatography with Pulsed Amperometric Detection
Mark R. Hardy, R. Reid Townsend, and Yuan C. Lee 54

Preparation of 6-^{125}I-Labeled Amiloride Derivatives
Dan Cassel, Miriam Rotman, Edward J. Cragoe, Jr., and Peter Igarashi 63

Detection of Biospecific Interactions Using Amplified Ellipsometry
Carl Fredrik Mandenius and Klaus Mosbach 68

High Pressure Liquid Chromatography Methods for Separation of ω- and (ω-1)-Hydroxy Fatty Acids: Their Applications to Microsomal Fatty Acid ω-Oxidation
Toshifumi Aoyama and Ryo Sato 73

Determination of Microsomal Lauric Acid Hydroxylase Activity by HPLC with Flow-Through Radiochemical Quantitation
Maria C. Romano, Kenneth M. Straub, Lee Ann P. Yodis, Regina D. Eckardt, and John F. Newton 83

Chemical Synthesis of Deuterated Folate Monoglutamate and *in Vivo* Assessment of Urinary Excretion of Deuterated Folates in Man
Jesse F. Gregory III and John P. Toth 94

Use of "Submarine" Gel Electrophoresis for Running Multiple Two-Dimensional Protein Gels *Suraj P. Bhat and Chandrasekhar N. Nagineni* 105

Rapid Identification of Clones Using the Same Degenerate Oligonucleotide Mixture for Both Screening and Sequencing *R. Nichols and Jack E. Dixon* 110

Separation of Calmodulin from Calcium-Activated Protein Kinase Using Calcium-Dependent Hydrophobic Interaction Chromatography
N. H. Battey and M. A. Venis 116

Free Malondialdehyde Determination in Human Plasma by High-Performance Liquid Chromatography *Claude Largillière and Serge B. Mélancon* 123

Separation of Large DNA Restriction Fragments on a Size-Exclusion Column by a Nonideal Mechanism
Barry E. Boyes, Douglas G. Walker, and Patrick L. McGeer 127
Determination of Avidin and Streptavidin by a Modified Bradford Method
Harmesh K. Sharma and Claude Tihon 135
A High-Precision Selective Determination Method for Free *N*-Acetylneuraminic Acid in Human Serum
Naokazu Sugiyama, Kin-ichi Saito, Hideo Mizu, Masayoshi Ito, and Yoshitaka Nagai 140
An Assay for Phentolamine Using High Performance Liquid Chromatography with Electrochemical Detection
Brent D. Kerger, Robert C. James, and Stephen M. Roberts 145
Application of ^{19}F Nuclear Magnetic Resonance to Examine Covalent Modification Reactions of Tyrosyl Derivatives: A Study of Calcineurin Catalysis
Bruce L. Martin and Donald J. Graves 152
Rapid Determination of Kinetic Constants by Competitive Spectrophotometry
Se Young Hwang, Karen S. Brown, and Charles Gilvarg 161
Protein Interaction with Immobilized Ligands: Quantitative Analyses of Equilibrium Partition Data and Comparison with Analytical Chromatographic Approaches Using Immobilized Metal Affinity Adsorbents
T. William Hutchens, Tai-Tung Yip, and Jerker Porath 168
A Stain for Iron-Containing Proteins Sensitive to Nanogram Levels of Iron
Che-Fu Kuo and Irwin Fridovich 183
Radioiodination of Proteins and Lipoproteins Using *N*-Bromosuccinimide as Oxidizing Agent
H. J. Sinn, H. H. Schrenk, E. A. Friedrich, D. P. Via, and H. A. Dresel 186
Optimal Conditions for Hybridization with Oligonucleotides: A Study with *myc*-Oncogene DNA Probes
Catrine Albretsen, Bjørn-Ivar Haukanes, Rein Aasland, and Kjell Kleppe 193
Protein Determination Using Bicinchoninic Acid in the Presence of Sulfhydryl Reagents *Hazel D. Hill and James G. Straka* 203
Purification of Forssman and Human Blood Group A Glycolipids by Affinity Chromatography on Immobilized *Helix pomatia* Lectin
B. Virginia Torres and David F. Smith 209
Amino Acid Analysis by Dinitrophenylation and Reverse-Phase High-Pressure Liquid Chromatography *Robert C. Morton and Gerhard E. Gerber* 220
Indirect Active-Site Titration of Plasminogen Activators *Ralph A. Magnotti, Jr.* 228
A Ligand–Receptor Binding Assay by Receptor Immobilization
Masao Takami, Izumi Kasuya, and Hajime Tsunoo 238
A Quantitative Evaluation of Promoter Activity by Microinjection of Chloramphenicol Acetyltransferase Hybrid Genes into Tissue Culture Cells
G. Buschhausen-Denker and H. H. Arnold 243
Phospholipase A2 Assay Using an Intramolecularly Quenched Pyrene-Labeled Phospholipid Analog as a Substrate
Tom Thuren, Jorma A. Virtanen, Pentti J. Somerharju, and Paavo K. J. Kinnunen 248
Routine Detection of Calcium-Binding Proteins following Their Adsorption to Nitrocellulose Membrane Filters *Maxwell T. Hincke* 256

High Performance Liquid Chromatographic Quantitation of Carboxypeptidase Activity Secreted by Human Hep G2 Cells
Brian G. Grimwood, Anthony L. Tarentino, and Thomas H. Plummer, Jr. 264

BOOK REVIEW

Methods in Enzymology, Vol. 144, *Structural and Contractile Proteins,* Part D, *Extracellular Matrix.* Edited by L. W. Cunningham Reviewed by *Beverly Peterkofsky* 269

NUMBER 2, MAY 1, 1988

Biocytin Hydrazide—A Selective Label for Sialic Acids, Galactose, and Other Sugars in Glycoconjugates Using Avidin–Biotin Technology
Edward A. Bayer, Haya Ben-Hur, and Meir Wilchek 271
Acidimetric Assay for Phospholipase A Using Egg Yolk Suspension as Substrate
Nget-Hong Tan and Chon-Seng Tan 282
An Affinity Gel for the Inhibition, Binding, and Isolation of Serine Proteases
Erik Millqvist, Hans Petersson, and Mats Rånby 289
The Quantitative Discrimination of Corneal Type I, but Not Skeletal Type II, Keratan Sulfate in Glycosaminoglycan Mixtures by Using a Combination of Dimethylmethylene Blue and Endo-β-D-galactosidase Digestion
J. Melrose and P. Ghosh 293
Reversed-Phase Ion-Pair Chromatography of Straight- and Branched-Chain Dicarboxylic Acids in Urine as Their 2-Nitrophenylhydrazides
Hiroshi Miwa and Magobei Yamamoto 301
Determination of Inositol Phosphates and Other Biologically Important Anions by Ion Chromatography *Robert E. Smith and Ronald A. MacQuarrie* 308
A High Pressure Liquid Chromatographic Method for the Separation and Quantitation of Water-Soluble Radiolabeled Benzene Metabolites
Patrick J. Sabourin, William E. Bechtold, and Rogene F. Henderson 316
Two Sensitive, Convenient, and Widely Applicable Assays for Marker Enzyme Activities Specific to Endoplasmic Reticulum *William A. Braell* 328
Preparation of Eumelanin-Related Metabolites 5,6-Dihydroxyindole, 5,6-Dihydroxyindole-2-carboxylic Acid, and Their *O*-Methyl Derivatives
Kazumasa Wakamatsu and Shosuke Ito 335
Purification of Transiently Transfected Cells by Magnetic Affinity Cell Sorting
Raji Padmanabhan, Christopher D. Corsico, Tazuko H. Howard, Wolfgang Holter, C. Michael Fordis, Mark Willingham, and Bruce H. Howard 341
A Method for the Determination of *N*-Acetylglucosaminyltransferase III Activity in Rat Tissues Involving HPLC
Atsushi Nishikawa, Shigeru Fujii, Toshihiro Sugiyama, and Naoyuki Taniguchi 349
Acetyl Coenzyme A Synthetase Catalyzed Reactions of Coenzyme A with α,β-Unsaturated Carboxylic Acids *Smita S. Patel and David R. Walt* 355
Protein Blotting with Direct Blotting Electrophoresis *Stephan Beck* 361
A Continual Spectrophotometric Assay for Amino Acid Decarboxylases
Fiona Scriven, Kenneth B. Wlasichuk, and Monica M. Palcic 367

Electrophoretic Determination of Fructose 6-Phosphate,2-Kinase
Yukari Kora-Miura, Shinya Fujii, Masafumi Matsuda, Yutaka Sato, Kohei Kaku, and Toshio Kaneko 372
A Bioluminescent Assay for the Determination of Phosphoenolpyruvate Carboxykinase Activity in Nanogram-Sized Tissue Samples *Monika Wimmer* 376
Purification of Residualizing Glycoconjugate Labels for Protein by Reversed-Phase High-Pressure Liquid Chromatography
John W. Baynes, Janet L. Maxwell, Kazi M. Rahman, and Suzanne R. Thorpe 382
Direct Determination of Urinary Pseudouridine by High-Performance Liquid Chromatography *Tetsuya Yamamoto, Kazuya Higashino, Shinsuke Tamura, Hiroshi Fujioka, Yoshiki Amuro, and Toshikazu Hada* 387
The Effect of Fast Protein Liquid Chromatography (FPLC) Cationic Exchange Purification on the Crystallization of a Monoclonal Fab Fragment
John D. Orbell, Luke W. Guddat, Ken J. Machin, and Neil W. Isaacs 390
Low Volume Processing of Protein Blots in Rolling Drums
Nicholas Thomas, Christopher N. Jones, and Philip L. Thomas 393
Enzymatic Determination of L-Phenylalanine and Phenylpyruvate with L-Phenylalanine Dehydrogenase *Werner Hummel, Horst Schütte, and M.-R. Kula* 397
A Semiautomated System for Measurement of 96 Simultaneous Spectrophotometric Enzyme Assays *Edward G. Butler, Rachhpal Gill, and Lisa Garych* 402
Nonlinear Estimation of Parameters in Biphasic Arrhenius Plots
Martin L. Puterman, Nina Hrboticky, and Sheila M. Innis 409
Adsorptive Transfer Stripping Voltammetry: Determination of Nanogram Quantities of DNA Immobilized at the Electrode Surface *Emil Paleček* 421
Measurement of Cyst(e)amine in Physiological Samples by High Performance Liquid Chromatography
Rolando A. G. Garcia, Lawrence L. Hirschberger, and Martha H. Stipanuk 432
A Chemical Method for Introducing Haptens onto DNA Probes
George H. Keller, Cecilia U. Cumming, Dao-Pei Huang, Mark M. Manak, and Robert Ting 441
Phenyldichlorophosphate as an Aid in Studies of Decarbamylation of Carbamylated Acetylcholinesterase *Raymond M. Dawson and Michael Poretski* 451
Heparin Requirement for the Quantitation of Fibrinogen Production by Primary Hepatocyte Cultures *Chi V. Dang, Frank M. LaDuca, and William R. Bell* 456
Exclusion Chromatography with Controlled-Pore Glass Beads to Isolate *Chlorella* Chromatin and Its Applications *Tsutomu Fukumoto, Takao Nishimura, Tadako Nagasawa, Katsumi Kitajima, and Tatsuichi Iwamura* 463
Receptor Analysis: An Arithmetic Correction Improves Precision and Accuracy
Günter Oettling and Hans Moeller 472
Electrophoresis of DNA Restriction Fragments in Poly-*N*-Acryloyl–Tris Gels
Branko Kozulić, Klaus Mosbach, and Maciej Pietrzak 478
Microassay of Heme Oxygenase by High-Performance Liquid Chromatography: Application to Assay of Needle Biopsies of Human Liver
B. C. Lincoln, A. Mayer, and H. L. Bonkovsky 485
Enhanced Detection of Glycoproteins in Polyacrylamide Gels
G. Muñoz, S. Marshall, M. Cabrera, and A. Horvat 491

Purification of Ornithine Aminotransferase by Immunoadsorption
Jon M. Leah, E. Ellen Billett, and Trevor Palmer 495
On-Resin Biotinylation of Chemically Synthesized Proteins for One-Step Purification
Thomas J. Lobl, Martin R. Deibel, Jr., and Anthony W. Yem 502
An Ultrasensitive Colorimetric Assay for Manganese
Wayne F. Beyer, Jr., and Irwin Fridovich 512
Synthesis of Fluorescent Oligosaccharides for Covalent Attachment to Living Cells
Carl G. Gahmberg and Martti Tolvanen 520
Fluorography of Tritium-Labeled Proteins in Silver-Stained Polyacrylamide Gels
Peter Kulcsár and Glenn D. Prestwich 528
Site-Directed Antibodies for Probing the Structure and Biogenesis of Phosphatidylinositol Glycan-Linked Membrane Proteins: Application to Placental Alkaline Phosphatase *C. A. Bailey, A. Howard, R. Mićanović, J. Berger, E. Heimer, A. Felix, L. Gerber, L. Brink, and S. Udenfriend* 532
A Complete Quantitative N-Terminal Analysis Method *Jui-Yoa Chang* 542

AUTHOR INDEX FOR VOLUME 170 557

The Subject Index for Volume 170 will appear in the December 1988 issue as part of a cumulative index for the year 1988.

INFORMATION FOR AUTHORS

Analytical Biochemistry is an international journal that publishes original articles on methods and methodology of interest to the biological sciences and all fields that impinge on biochemical investigation. In addition to the expected techniques that apply to biochemical preparations and analysis, the following should be considered as within the scope of the journal:

— Cell biology and cell, tissue, and organ culture methods that are of general application.

— Membranes and membrane proteins. These methods form a major section of interest.

— Procedures of interest in the field of molecular genetics including those necessary for cloning, sequencing, and synthesis of nucleic acids and for mutagenesis.

— Purification of enzymes and other proteins, but only if the methods are both novel and applicable generally, i.e., the interest is in a means of purification rather than in the protein that is being purified.

— Immunological techniques both analytical and preparative, if they are of general applicability by biochemists. Immunoassays are included but only if a unique approach is introduced rather than the determination of a substance not previously assayable.

— Pharmacological and toxicological research techniques that offer a biochemical approach.

— Review articles dealing with timely topics. Although these are generally invited, unsolicited outlines or manuscripts will be considered.

Manuscripts are accepted for review with the understanding that the same work has not been and will not be nor is presently submitted elsewhere, and that its submission for publication has been approved by all of the authors and by the institution where the work was carried out; further, that any person cited as a source of personal communications has approved such citation. Written authorization may be required at the Editor's discretion. Articles and any other material published in *Analytical Biochemistry* represent the opinions of the author(s) and should not be construed to reflect the opinions of the Editor(s) and the Publisher.

Authors submitting a manuscript do so on the understanding that if it is accepted for publication, copyright in the article, including the right to reproduce the article in all forms and media, shall be assigned exclusively to the Publisher. The Publisher will not refuse any reasonable request by the author for permission to reproduce any of his or her contributions to the journal.

Address. Manuscripts should be submitted in quadruplicate to *Analytical Biochemistry*, Editorial Office, Seventh Floor, 1250 Sixth Avenue, San Diego, California 92101. In the case of rejected manuscripts, only one copy of the manuscript will be returned to the author.

An extended Information for Authors is published in each July issue. Reprints are available from the Editorial Office. Authors should consult this Information for details on nomenclature, IUPAC-IUB rules and recommendations, and accepted abbreviations.

Manuscripts should be concise and consistent in style, spelling, and use of abbreviations. In order to facilitate the processing of manuscripts, the journal requests that at least four copies be submitted, each copy to include all figures (originals or glossy prints) and tables. The original copy must be typewritten, double-spaced, on one side of white bond paper, about $8\frac{1}{2} \times 11$ inches in size, with one-inch margins on all sides. Each manuscript should have a separate title page noting title of article, authors' names (without degrees), a running title (not exceeding 55 letters and spaces), an abstract (250 words), the address to which proof should be mailed, and the corresponding author's telephone number and fax or telex number. In creating a title for your paper, please omit words such as *novel*, *rapid*, *improved*, *simple*, *sensitive*, *efficient*, *convenient*, *new*. Each is felt to be redundant and actually slows down the reader who is scanning the article titles and wants to know more about the method itself. Please substitute informative words that will give readers a more complete idea of the usefulness of your paper with regard to the limitations of their equipment or sample type. For example, what *kind* of method are you describing? Abbreviations should not be used in the article title or running title.

Please number all pages of the manuscript.

Authors should indicate the following on the title page: (1) the appropriate subject category for listing in the Table of Contents; (2) six descriptive key words for computer-aided selection of reviewers. (Please see the Appendix at the end of the extended Instructions to Authors published in each July issue for a list of recommended key words.) These words will also be used in the preparation of the annual cumulative subject index for the journal.

Text. The suggested organization of an article is: abstract; introductory statement; Materials and Methods; Results; Discussion; Acknowledgments; References. Some of these sections may be combined if the presentation is thereby made clearer or more effective. Although this is the general form, the journal will accept short papers of approximately two printed pages that are arranged without specific sections except for a short abstract.

Names of chemical or organic substances should follow the recommendations of the IUPAC–IUB Combined Commission on Biochemical Nomenclature (CBN) as published in the extended Instructions for Authors, appearing in the July issue of this journal.

Authors should draw attention to any particular chemical or biological hazards that may be involved in carrying out the experiments described. Any relevant safety precautions should be

described; if an accepted code of practice has been followed, a reference to the relevant standards should be given.

Figures should be numbered consecutively with Arabic numerals in order of mention in the text: each figure should have a descriptive legend. Legends should be typed together on a separate sheet, double-spaced. All illustrations should be in finished form ready for photoreproduction. A convenient size for drawings is $8\frac{1}{2} \times 11$ in. (21×27.5 cm). Drawings should be made with India ink on tracing linen, smooth surface white paper, or Bristol board. Graph paper if used should be ruled in blue. Grid lines that are to show in the final reproduction should be inked in black. Glossy prints properly prepared are acceptable.

Photographs must be kept to a minimum. All photographs should be submitted as single glossy prints with strong contrasts and not mounted on cardboard; the magnification should be indicated by a scale where possible. **Simple histograms should be avoided. A table or a paragraph in the text is preferred.**

Illustrations in color can be accepted only if the authors defray the cost.

Tables should be typed on separate pages, numbered consecutively with Arabic numerals in order of mention in the text. Each table should have a short explanatory title.

Authors may wish to submit complex tables as camera-ready copy. In this way, they are spared tedious proofreading and the chance of error is reduced. Tables submitted as camera-ready copy should be typed single-spaced whenever possible, and preferably submitted as 5×7-in. glossy prints.

Footnotes should be designated in text by superscript numbers and typed on a separate sheet. Footnotes to tables should be identified with lowercase italic superscript letters and placed at the bottom of the table.

References to the literature should be cited in the text by Arabic numerals in parentheses and listed at the end of the paper in consecutive order. Abbreviations of journal titles should follow the style used in *Chemical Abstracts Service Source Index,* 1985. Please note style of capitalization and punctuation for journal articles and edited books in the following examples:

1. Jackson, G. E. D., and Young, N. M. (1987) *Anal. Biochem.* **162,** 251–256.
2. Bovey, F. A., and Jelinski, L. W. (1983) Chain Structure and Conformation of Macromolecules, p. 150, Academic Press, New York.
3. Britton, G. (1985) *in* Methods in Enzymology (Law, J. H., and Rilling, H. C., Eds.), Vol. 111, pp. 113–149, Academic Press, Orlando, FL.

Proofs. Proofs will be sent to the authors with reprint order forms. Fifty reprints of each article are provided free of charge. Additional reprints may be ordered using the form provided.